EXPERIMENTS WITH FROG'S EGGS.

T. H. MORGAN.

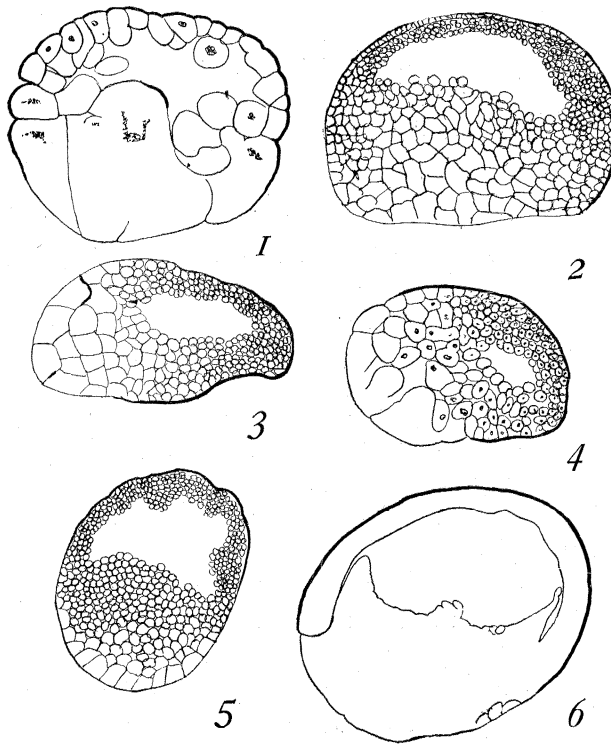
The following experiments were carried out in part during the spring of 1905, and in part during the present year. As the headings of the different sections indicate I have brought together the results of experiments of very different kinds, but since they all bear on the same questions it seemed preferable to put the results together in one paper, rather than to scatter them through several. The following topics are discussed: (1) The Development of the Frog's Egg out of Water, (2) The Increase in the Size of the Egg during the Segmentation Stages, (3) Obliteration of the Blastocœl by means of a Centrifugal Force and the Effect on the Subsequent Development, (4) Removal of the Roof of the Blastocœl, (5) Effects of Cold on the Early Development, (6) The Early Development of the Lithium Larvæ of the Frog, (7) Effects of Lithium Chlorid and Sodium Chlorid Acting Together, (8) Effects of Lithium Chlorid and Magnesium Chlorid Acting Together, (9) The Chemical Versus the Osmotic Effects of Salt Solutions.

The Development of the Frog's Egg Out of Water.

In order to determine whether the segmentation cavity of the frog's egg is simply a water-filled space, left by the cells of the blastula as they separate, or whether the cavity is formed by the active secretion of the surrounding cells, I placed eggs on pieces of filter paper and allowed them to develop out of water. On the first assumption, the blastocœl is filled with water, that, percolating *between* the cells, passes into the interior. The enlargement of the normal egg during the cleavage period shows that water is really absorbed by the egg, but whether this water simply fills the enlarging segmentation cavity, or whether it enters the cells could only be determined by keeping the eggs out of water during the early development.

In the first experiment the eggs were placed on pieces of wet filter paper after their outer membranes had been removed. The

lower end of the paper simply dipped into water. Under these conditions the eggs developed normally and young tadpoles appeared. The eggs may have absorbed enough water from the filter paper for their normal development. In later experiments, the eggs, freed from their outer mem branes, were placed on dry pieces of filter paper, six to eight on each small piece. The water adhering to the inner membranes made a small damp spot around each egg. The pieces of paper were then put into glasses with covers to prevent further drying. In the course of a few hours the eggs became flattened on the side in contact with the paper. The segmentation continued under these conditions, and



FIGS. 1–6. Eggs developing out of water.

in a few cases, noticeably those where too much water had been left, normal or abnormal embryos developed. In the majority of cases, however, the egg died, or the development was retarded

in the late cleavage stages, the amount of desiccation determining the result. Despite the drying of the egg with its accompanying flattening, the segmentation cavity appeared in it, as sections showed, and while it generally failed to reach the proportions characteristic of normal development (except when the paper was too wet), yet that it should develop at all under the adverse conditions of the experiment, demonstrates that its contents are produced, in part at least, as a secretion of the surrounding cells. A few sections of these eggs are reproduced in Figs. 1–12.

A section through an egg that had been placed on a dry piece of filter paper at the two-cell stage, and killed some hours later, is shown in Fig. 1. A large segmentation cavity is present in the interior although the egg is flattened below from pressure.

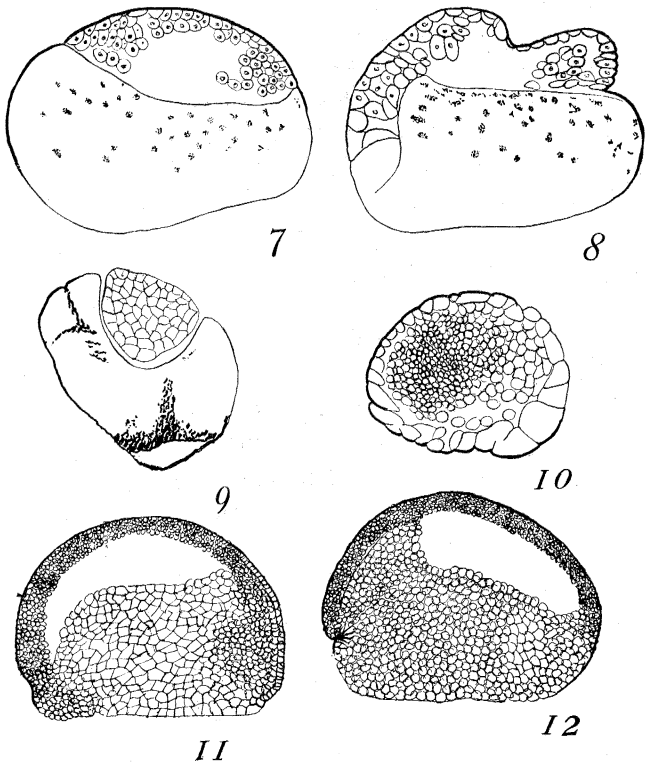
A later stage is shown in Fig. 2. The egg was attached by its white pole. The blastocœl is large and not very different from that of a normal egg. A still later stage of an egg, that was still further compressed (on one side in this case), is shown in Fig. 3. The segmentation cavity is smaller than in the normal blastula of this age. The reduction is probably connected with the great flattening of the egg. Similarly in the next figure, Fig. 4, the segmentation cavity is reduced in size. In the next two figures, Figs. 5 and 6, the segmentation cavity is larger, and as large, in fact, as that of the normal egg. These eggs were but little compressed, and, therefore, developed more nearly in the normal manner.

The next two figures, Figs. 7 and 8, show a different type of development. The yolk has been so much injured by the drying, that it has failed to segment, yet it is filled with nuclei each surrounded by pigment. Both of these eggs were fastened by the lower hemisphere. The tops of the eggs were less injured, and had divided into small cells, that arch over a large segmentation cavity.

The next egg is very different, Fig. 9. Here, too, the yolk has been killed, but there is present a solid mass of cells imbedded in the yolk. The egg seems to have been attached to one side of the black hemisphere, and the small cells in the interior are due to the shifting of the interior of the egg. The next figure, Fig. 10,

shows a somewhat similar condition, where, however, the lighter material of the top of the egg has sunken into the interior. This was due to the eggs being placed on the paper in an inverted position while in the two-cell stage. The figures show that the smallest cells are in the interior of the egg, and not on the surface of the black hemisphere.

In order to meet the possible objection that the egg may absorb water from the small, slightly damp, piece of paper, another series



FIGS. 7–12. Eggs developing out of water.

of experiments was carried out, in which the eggs, deprived of their outer coats, were kept on pieces of glass. For several minutes the eggs were left on the glass exposed to the air, in order that the water sticking to their outer coats, or to the glass, might dry off. They were then placed in the moist chamber. An egg that had reached a late blastula stage under these conditions is

shown in Fig. 11, and another at the beginning of gastrulation in Fig. 12. Both show a large segmentation cavity. The results may be summed up as follows: If the egg in the two- or four cell-stage is taken from the water and kept from drying, it will develop, and the segmentation cavity will be formed, which, although sometimes smaller than the normal, if the egg is very dry, yet its presence under these adverse conditions shows that it must be due to a secretion poured out by the blastula cells, and that it is not due directly to the passage of the water from the outside into the egg.

I have found that the eggs of *Fundulus heteroclitus* will also develop out of water if simply placed on a glass plate in a moist atmosphere. The eggs of the starfish will pass through several segmentation stages under similar conditions, but so little water remains on the glass that it evaporates quickly and the eggs are so delicate that they cannot withstand the drying. The blastula becomes flattened and is nearly solid, but on being placed again in sea water it quickly rounds up, and the segmentation cavity appears. The normal segmentation cavity in the starfish is very large and is early formed. Its absence in the eggs out of water may be due to the flattening of the egg, but possibly its absence is due to the necessity of the cells to absorb water in order that it may be formed. Those who have studied the segmentation of the sea urchin egg in water under compression, as when it is placed between compressing plates, have observed the absence of the segmentation cavity, even when the segmenting egg has become two-layered. In the frog also, as we have seen, the compression may be responsible for the suppression of the full development of the blastocœl, but since the compression is not carried so far in the latter case, the formation of the cavity is not suppressed, and the fact that it forms at all under these adverse conditions goes to show that its origin is due to the activity of the surrounding cells.

The Increase in the Size of the Egg During the Segmentation Stages and the Increase of the Blastocœl.

I measured some eggs of *Rana sylvestris* at different stages of development in order to see whether the increase in size of the

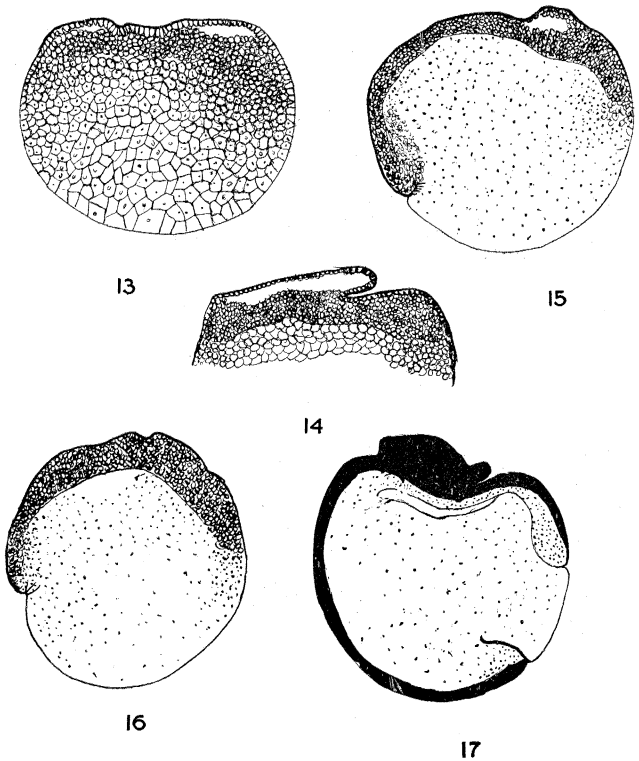
egg during the segmentation stages and pregastrula stages is equal to or greater than the space of the blastocœl. On account of the variation in size of different eggs it would have been better to measure the same egg at different times. I shall give some measurements of several eggs of the same bunch but not the same eggs. In one case the unsegmented eggs measured 5.1, 5.2, 5.3 and 5.4. The average eggs seem about 5.3. At the beginning of gastrulation the eggs measured 5.6 and 5.7. When the dorsal lip was widely horseshoe-shaped the eggs measured 5.6 and 5.8; and when the gastrula lips were nearly close 5.8 and 5.9. In another case the unsegmented eggs measured 5.2, 5.3, 5.4 and at the beginning of gastrulation 5.5, 5.6, 5.7. In another case the unsegmented eggs measured 5.2, 5.3, 5.4, and at the beginning of gastrulation 5.4, 5.5, 5.8.

If we assume that the unsegmented egg measures 5.3 and that the egg about to gastrulate measures 5.6, the latter will have gained about two ninths of the volume of the former, or roughly one fourth. An approximate estimate of the size of the blastocœl when fully formed, as compared with the blastula in which it is contained, shows that the blastocœl is about one eighth the entire volume. Hence, while the egg has gained one fourth in volume, only one eighth of its increase is due to the segmentation cavity; *i. e.*, only one half of the increase in size can be accounted for by the segmentation cavity alone, and the other half must have been due to the absorption of water by the cells of the blastula. Thus we must conclude that the egg is absorbing water during the segmentation stages, and that at the same time it is giving up to the blastocœl an amount of fluid that is approximately half of the amount absorbed. If the egg is placed under conditions where it can not absorb water, it gives up, nevertheless, nearly the normal amount to the blastocœl.

Obliteration of the Blastocœl by Means of a Centrifugal Force and the Effect on Subsequent Development.

The eggs of *Rana sylvestris* in a late blastula stage were put into tubes of water and revolved on a small centrifuge at the rate of 1,600 revolutions per minute for ten minutes. At the end of

this time the black hemisphere of the egg was much flattened, and sections show, Fig. 13, that the fluid of the blastocœl has been completely driven out of the egg. The figure shows that not only has the top of the egg been flattened, but the small cells at the sides of the blastocœl have moved over across the top of the yolk, so that a thick layer of small cells lies as a flat plate on top of the larger yolk cells. Here and there a small crack or space is present along the region of contact of the large and the small cells. Not infrequently the top of the egg is thrown over as a fold, as shown in Fig. 14. This is due no doubt to the fact that as an arch it occupies more space than when flattened down on the egg. In the preserved eggs an artefact often appears beneath the outer layer of cells.



FIGS. 13–17. Eggs of *Rana sylvestris* after having been rotated at 1,600 revolutions per minute. 13, immediately after rotating; 14, top of another egg at same time; 15–17, later stages when gastrulation has begun.

These eggs gastrulated and produced embryos. Sections through several of the intermediate stages show that, after the removal from the machine, the top of the egg did not regain its former roundness, Fig. 15, but remained flattened. In a few cases a small space was found near the top of the egg beneath the ectoderm that may represent a part of the blastocœl, Fig. 16. A later stage is shown in Fig. 17. The blastopore is closing, the archenteron is present, but no indications of the blastocœl can be found. A lump of ectoderm lies near the anterior end of the embryo. It is an almost constant feature of these embryos, and owes its origin to the injury of the roof of the blastocœl. Its location does not necessarily mean that the normal embryo extends to the top of the egg, because extensive movements of the cap of small cells must take place during the time of gastrulation. Nevertheless it is true that this lump of cells originated near the top of the egg, and had been carried downwards towards the anterior end of the embryo. In fact the material of which it is composed may represent the ectoderm of the anterior end that had been carried upwards by the action of the centrifugal force.

The results show that the blastocœl is not essential for the formation of the frog embryo, since the process of gastrulation may take place in its absence. This does not mean that the blastocœl may not be made use of in ordinary development; in fact it is made use of, since the yolk mass is thrown into it, but the result does mean that the blastocœl is not essential for development. Two methods of interpreting the blastocœl have been followed by embryologists. The commonly accepted method is to "explain" it by assigning to it a purpose. Its purpose is to make a space into which the yolk cells can be thrown at the time of gastrulation. It would seem from this point of view that the blastocœl is not only a useful, but also an essential organ in development. The results show, however, that this is not the case.

The other method of interpretation is that of the school of developmental mechanics which has tried to account for the formation of the blastocœl as the results of some such mechanical process as infiltration, and have assigned it to the function of producing an osmotic pressure on the walls of the blastula. Rhumbler

has tried to account for the gastrulation process as the outcome of the accumulation of certain waste products in the blastocœl, but since the development of the gastrula may take place when no blastocœl is present, this explanation of the mechanics of gastrulation does not appeal to me as a probable one.

Removal of the Roof of the Blastocœl.

It has been suggested by Rhumbler that the process of gastrulation may be due to the accumulation of waste products, carbon dioxide for instance, in the blastocœl fluid. The presence of such a substance would bring about changes in the surface tension on one side of the yolk cells, which, by causing them to change shape, is imagined to bring about the inturning of the cells. It seemed to me that this view might be tested by emptying the blastocœl of its fluid before gastrulation had occurred. If the inturning still took place the result would show that the process need not be connected with the blastocœl fluid, or with substances that have become dissolved in it.

There was also another question that I wished to examine by means of the same experiment. The formation of the large blastocœl space takes place at the time when the embryo-forming materials, that come from the upper hemisphere, are moving outwards and downwards at the sides of the blastocœl, and the question arises whether this movement is connected with the development of the blastocœl space. Finally there is still a third question involved, namely, whether the movement of the material is due to the downward pressure of the cells themselves of the roof of the blastocœl.

In the first set of experiments the roof of the blastocœl was opened, and injured by plunging a needle into it. Despite the operation the process of gastrulation still took place in most cases, and a normal embryo developed. In consequence of the operation, as sections show, a large part of the fluid of the blastocœl is set free, although a small part of it may remain. When the operation is carried out at an early stage the cavity may develop later and suffice to bring about the gastrulation, if it were really due to this factor. In order to meet this possible objection, I operated on two other sets of eggs, one at the time when the

blastocœl had reached its highest point of development, and the other at the time when the dorsal lip of the blastopore had just appeared on the surface. Sections showed that the blastocœl was emptied in large part, yet in both cases gastrulation took place. There is, however, to be noticed a distinct retardation in the time of gastrulating between the normal and the injured eggs. The hole made in the roof closes almost at once, but a lump of cells generally indicates, throughout the gastrulation process, the place of injury. Owing to the closure of the top, the pressure relations of the cells will be again largely reëstablished, but the delay in the time of gastrulation indicates that the injury has to some extent interfered with the processes involved in the act of gastrulating.

The results show that Rhumbler's hypothesis is probably incorrect at least so far as the blastocœl is concerned. If however he should shift his position and assume that it is the accumulation of waste substances in the interior of the egg itself, *i. e.*, in the middle of the yolk-cells, that causes the invagination, the results are not fatal to his view. I attempted therefore to test Rhumbler's hypothesis in another way. If the amount of carbon dioxide outside the egg can be made equal to that in the interior, the process of gastrulation should not occur, if Rhumbler's view is correct. I placed frog's eggs in the late blastula stages in a bottle containing some water. By means of a tube running beneath the water I forced air that I had held in my lungs for a few seconds through this water. The air above the water was also displaced through an outlet. The communications with the outside were then closed. It seemed probable that there would be as much, and probably a great deal more carbon dioxide outside the eggs than inside, yet during the following six to twelve hours the gastrulation took place in the normal manner. This result also is not favorable to Rhumbler's interpretation. Other observations and experiments have led me to think that the process of gastrulation cannot be explained by such mechanical processes as surface tension, and I have tried to show elsewhere [1] that the change in shape of the cells that leads to the invagination is due to a process of active contraction of the cells, which

[1] *Raux's Archiv*, XIX., 1905.

in turn is the outcome of the pressure relations of the cells on each other.

Effects of Cold on the Early Development.

In a preceding paper[1] I have described some abnormal embryos of the frog that were produced as the result of cold. The early segmentation stages of these eggs were not preserved, but I can now make good this deficiency by means of a series of young stages kept under similar condition (1° to 2° C.), that have been put up for me by Dr. N. M. Stevens.

The most obvious effect of the cold is of course to delay the development. The secondary effects involve (1) greater injury to the yolk cells than to those of the upper hemisphere, (2) effects on the formation of the segmentation cavity, and (3) in some cases the retention of the small cells at the upper hemisphere. How far this latter change may be directly connected with the injury to the lower cells, so that they fail to draw inwards, is a point difficult to determine, but if the cold is responsible for the failure of these cells to draw inwards, the retention of the small cells at the upper pole may directly result.

The details of the development of these eggs, left on the ice for eleven days, are as follows (the eggs were put on the ice April 9, at 10 a. m.):

April 9, 1 p. m. Some eggs still in the four-cell stage, others going into eight cells.

April 9, 6 p. m. Eight-cell stage. Small segmentation cavity present.

April 10, 9 a. m. Some eggs going into the twelve-cell stage. The segmentation cavity is well developed (Fig. 18).

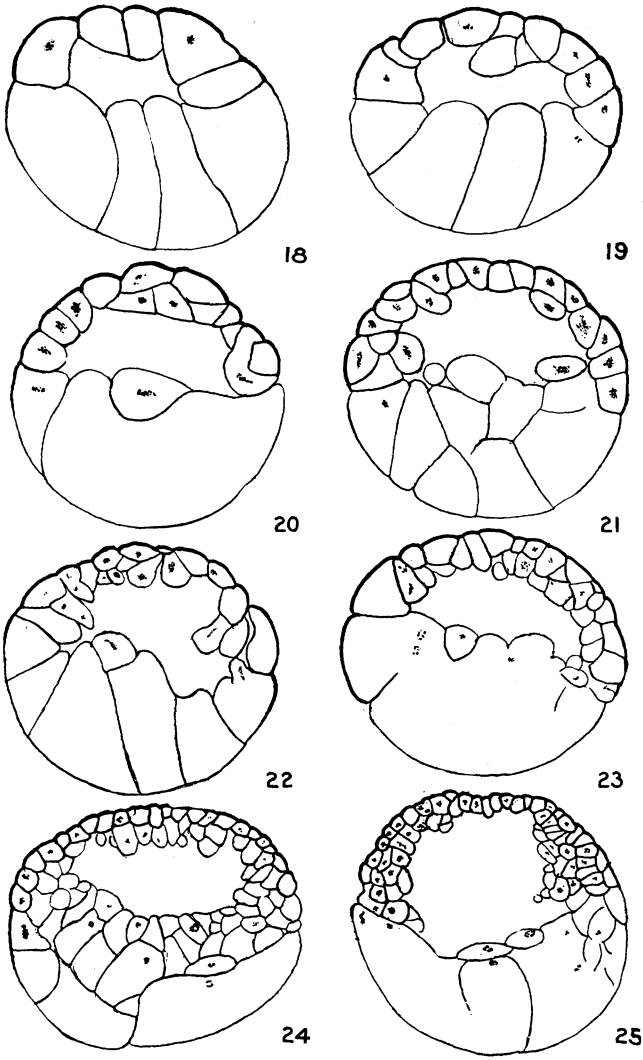
April 10, 5 p. m. Sixteen-cell stage. The segmentation cavity extends far down into the yolk.

April 11, 9 a. m. Another cell division has taken place. A large segmention cavity has developed (Fig. 19).

April 11, 8 p. m. Immigration of cells at the top of the egg taking place. Segmentation cavity better developed on one side than on the other.

[1] *Roux's Archiv*, XIX., 1905.

April 12, 9 a. m. Further division has occurred. Black cells are noticeably confined to the upper third of the egg, *i. e.*, there is not the normal downgrowth (Fig. 20).



FIGS. 18–25. Segmentation stages of egg kept on ice. *Rana palustris.*

April 12, 6 p. m. Condition same as last. Segmentation cavity large.

April 13, 9 a. m. Same condition; possibly another division has occurred.

April 13, 6 p. m. Some of the eggs have divided further. Large blastocœl (Fig. 21).

April 14, 9 a. m. Same as last.

April 14, 5:15 p. m. A large and irregular segmentation cavity, lying more on one side, is seen in sections (Fig. 22).

April 15, 9 a. m. The surface of the egg shows white flecks, and the cells have divided further. Large blastocœl present.

April 16, 2 p. m. Same. Cells irregular. Segmentation cavity large.

April 18, 9 a. m. Same. Large segmentation cavity. No downgrowth of small cells. Yolk irregularly divided (Fig. 23).

April 20, 9 a. m. Divided further. Top of egg brown instead of black. Large and irregular segmentation cavity (Fig. 24).

April 22, 9 a. m. In the surface view the outlines of the upper dark cells are very irregular. Large segmentation cavity surrounded by irregular cells (Fig. 25).

Compared with the normal set,[1] put up at the same time, the retardation of this cold series is apparent. After eleven days these eggs on the ice did not develop further than those at room temperature in twenty-four hours. Not only is there a delay, but the development has become quite abnormal, especially during the later stages. The sections show that the cells have in general an irregular outline, and are not compacted together as in the normal embryo. There is no downward migration of the material of the top of the egg, hence the abnormal forms of embryos described in my former paper. Great differences and abnormalities are found in the formation of the segmentation cavity, and this same difference was noticeable in the older stages. These older stages have been described in my earlier papers.

The Early Development of Lithium Larvæ of the Frog.

In a previous paper[2] I have described some of the effects of lithium solutions on the development of the frog's egg. The

[1] The control normal set are those described in my forthcoming paper on "The Origin of the Embryo-Forming Materials in the Frog's Embryo," 1906.

[2] *Roux's Archiv*, XVI., 1903.

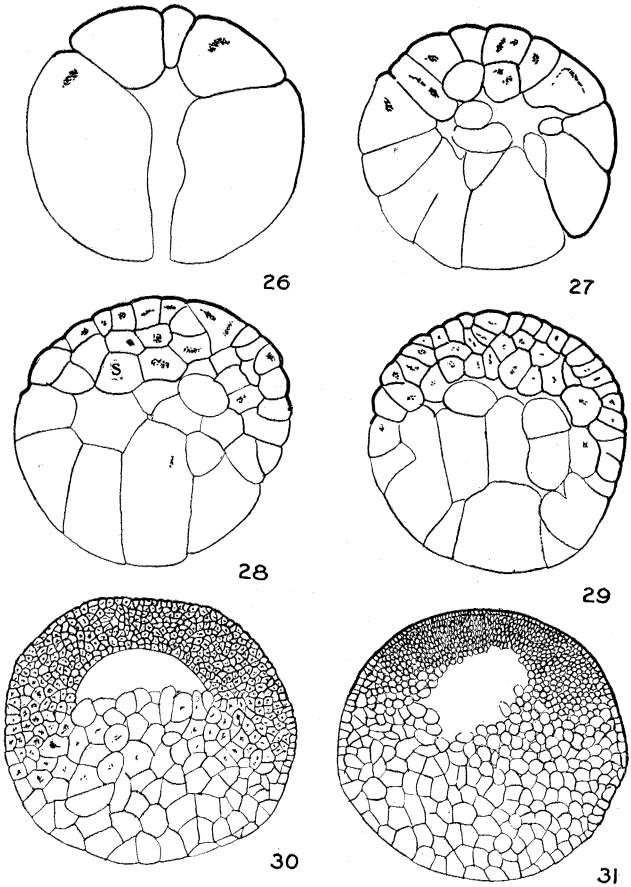
most peculiar and interesting type of these larvæ is that in which the embryo is formed in the interior of the egg instead of on the surface. In other cases the material of the top of the egg accumulates as a solid black cap upon which the anterior end of the neural plate sometimes appears. Embryos also appear in which the dark cells extend to different distances over the yolk.

The material that I used to study these embryos lacked the earlier stages, and while the observations on the living eggs left little doubt that the results were due to the failure of the upper cells to move downwards over the yolk, yet the actual details were not known. Since the question of the origin of the embryo-forming material is involved in my interpretation of the results it was desirable to obtain these missing stages. Dr. N. M. Stevens has kindly put up for me the necessary material for studying these missing stages of *Rana palustris*, the same species that I formerly used.

The eggs in the two-cell stage were put into a 0.5 per cent. solution of lithium chlorid and preserved at intervals of two hours. After the first two hours the eggs were in the eight-cell stage. A section through one of these is represented in Fig. 26. It differs little from a normal egg.[1] The segmentation cavity is somewhat smaller than that of the normal egg at this time. Two hours later the egg had reached the condition shown in Fig. 27. The inpulling of the cells of the upper hemisphere has begun. The segmentation cavity is noticeably small. Two hours later, Fig. 28, the divisions have gone further, and the upper cells now form almost a solid cap around the upper pole. A very small segmentation cavity, *S*, is present. Two hours later, Fig. 29, the smaller cells at the top still remain in place. A flattened segmentation cavity is present, but much reduced as compared with that of the normal egg. During the next four hours the changes are not marked. The small cells remain at the top and the segmentation cavity remains small. Two hours after this, *i. e.*, six hours after the last stage figured, the eggs are in the condition represented in Fig. 30. The roof of the segmentation cavity is very thick and the small cells still remain in the upper hemisphere. Four hours later, Fig. 31, the material at the top of the

[1] In preserving the lower blastomeres became separated in the egg.

egg has concentrated in a cap. It represents all of the ectoderm of the embryo. This brings the development to the stage at which my former series begins. If, after this stage is reached, the ectodermal material at the top of the egg turns into the interior, instead of growing down over the surface, as in the normal egg, an invaginated embryo is formed.



FIGS. 26–31. Segmentation stages of egg in lithium chloride. *Rana palustris.*

If it fails to turn in, it remains as a solid cap at the top, and if not too condensed the anterior end of the neural plate develops.

In the lithium types there is rather a sharp line of demarcation between ectoderm, mesoderm and endoderm. The three layers

appear to be more or less stratified in the order just given. This is best seen in the inverted type, in which the ectoderm turns in at the top. The mesoderm forms a sheet of cells wrapped around the neural plate, and the endoderm is drawn upwards as a sheet of cells over the mesoderm. (See Fig. I, Pl. XXIV., in my paper of 1903).[1] The endodermal layer, that is drawn upwards, appears to be that which is normally turned in around the lips of the circular blastopores. In addition to this there is always formed a tube opening on the surface that is lined by yolk cells. The opening of this tube lies near the equator of the egg and forms a crescent-shaped mouth there. The cells that line this tube correspond, I think, to those that make the anterior end of the normal archenteron. This part of the archenteron of the normal embryo appears to develop by the drawing apart of the yolk cells.

The mesoderm of the lithium larvæ is made up of the cells that lie just beneath the ectodermal cap. If we may draw any conclusion from the condition of the mesoderm in the lithium forms in regard to the origin of the first formed mesoderm of the normal embryo, we would conclude that it does not come from those cells of the 32- to 64-cell stages that are drawn beneath the surface at the top of the egg, but from cells lower down at about the level of the upper portions of the lower four blastomeres of the eight-cell stage. There may be, however, not a little latitude in respect to the potency of many of these cells, and their location is probably also a factor in their differentiation.

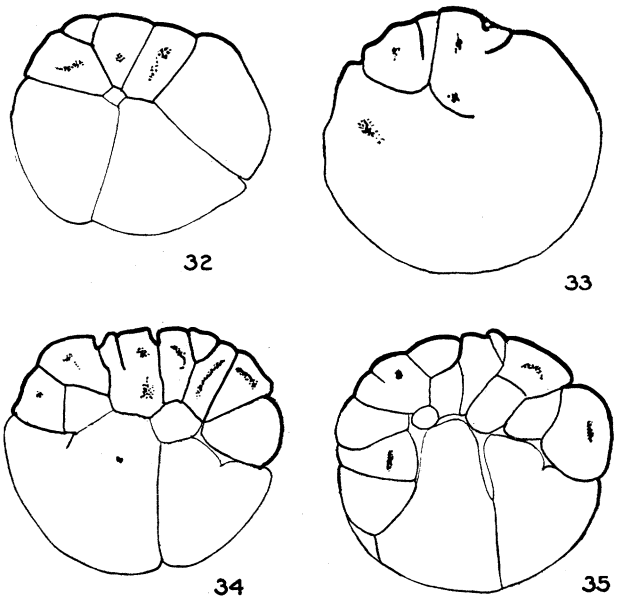
Effects of Lithium Chlorid and Sodium Chlorid Acting Together.

The effect of lithium seems to depend in large part upon chemical, rather than upon osmotic, effects. Other salts also act chemically in somewhat the same way as lithium, but to a less degree. I have tried the effects of these salts acting together and also separately on the eggs of *Rana palustris* and of *Bufo lentiginosus*. The material for the former species I owe to Dr. Stevens.

A solution of LiCl 0.5 and NaCl 0.6 per cent. gave the results shown in Figs. 32 and 33. The cleavage was not only greatly

[1] *Roux's Archiv*, XVI.

retarded but more irregular than normal, and came to an end before many divisions had been carried out. The details of the results were as follows: —



FIGS. 32–33. Segmentation of egg in lithium chloride and sodium chloride. *Rana palustris.*

FIGS. 34–35. Segmentation of egg in lithium chloride and magnesium chloride. *Rana palustris.*

The eggs in the two-cell stage were put into the double solution. After two hours a normal looking two-cell stage was present. After another two hours the cells had divided, and were in the same condition two hours later, Fig. 32. The egg shows little or no segmentation cavity. Other eggs have an abnormally small segmentation cavity. After two hours more, the upper cells have again divided, and the upper ends of the lower cells also. After two hours more, the eggs appear to be in the same condition, and this is also true for the next six hours. Sections of these eggs show a tendency for the blastomeres to run together (Fig. 33), and each contains several nuclei. After a further interval of eight hours, the cell-walls have largely disappeared on the surface.

The results show very clearly that the double solution has acted more injuriously on the egg than the lithium chlorid alone. The results appear, from a comparison with sugar solutions, not to be entirely due to the increase in osmotic pressure, but to the greater effect of the combined action of the sodium and the lithium.

Effects of Lithium Chlorid and Magnesium Chlorid Acting Together.

Eggs in the two-cell stage were put into a solution containing LiCl 0.5 + $MgCl_2$ 0.6 per cent. After two hours the eggs were still in the two-cell stage. Two hours later the eggs were in the 16-cell stage, some of them having divided with great irregularity. Sections show a small segmentation cavity present. Two hours later, Figs. 34 and 35, one or two further divisions have taken place. As shown in these figures the egg is nearly solid, and in other eggs there is no trace of the segmentation cavity. Another division has taken place after two hours more, and another division after another two hours. At this time the sections show the interior cell-walls running together to produce polynuclear cells. Two hours later the egg is as before, or has divided again, and after another four hours further division has occurred, but in this and the following stages (after eight hours), the cells especially the yolk cells are becoming polynuclear and division is apparently near its end without having produced a large number of cells. It is noticeable that the yolk cells have been more affected than the smaller cells of the upper hemisphere. The results are similar on the whole to those with LiCl + NaCl, but less marked; the younger stages being more regular. The segmentation cavity appears to have been practically obliterated.

The Chemical versus th Osmotic Effects of the Salt Solutions.

In order to determine, if possible, how far the action of the salt solutions is due to chemical effects and how far to osmotic effects, the following experiments were carried out with the eggs of *Rana sylvatica*. Eggs in the two-cell stage were put into solutions

of graded strengths. The amount of salt is given in percentages although this is not quite accurate, since, in the first place, one per cent., for example, is one gram added to 100 cc. (and not to 99 cc.) of water; and in the second place a certain further decrease in strength is caused by the water in the jelly around the eggs.

It is not an easy matter to determine definitely where normal development ceases, for, not only is the development often stopped at different stages in the same solution, but there is also a considerable overlapping in the different solutions. I have arbitrarily selected as the upper limit, the strength of solution in which, although the cleavage may continue for a time, gastrulation does not occur.

For LiCl alone it was found that gastrulation may take place and a normal embryo appear in a 0.5 per cent. solution. In a 0.55 per cent. solution the blastopore is large, its closure is delayed, and even prevented. In a 0.6 per cent. solution the blastopore appears, but generally no embryo develops; while the upper limit seems to be about 0.65 per cent. where practically all of the eggs fail to pass beyond the late segmentation stages. For NaCl the upper limit is above 2.0; but owing to an accident the upper limit was not accurately determined.

In a solution of LiCl 0.4 per cent. + NaCl 0.3 per cent. only the late segmentation stages appeared. Thus both solutions, being lower than the maximum for each, produce an injurious effect. In another double solution of LiCl 0.5 per cent. + NaCl 0.5 per cent. a few nearly normal embryos developed, but in a solution of LiCl 0.5 per cent. + NaCl 1.0 per cent. only the late segmentation stages developed, or rather a cap of black cells appeared at the top of the egg (surrounded in some cases by a band of gray cells, as in lithium larvæ that I formerly described). In a solution of LiCl 0.5 per cent. + NaCl 1.5 per cent. only late segmentation stages developed. This then may be taken as the limit.

A solution of cane sugar must be quite strong in order to prevent the development of the embryo. Even a 6.0 per cent. solution gives normal embryos. An 8 per cent. delays the closure of the blastopore, and a 10 per cent., while not preventing the appearance of the blastopore, does prevent its closure,

and no embryo develops. In a 12 per cent. solution the egg reaches only the late cleavage stages.

In a solution of LiCl 0.4 per cent. + sugar 5.0 per cent. only about the sixty-fourth-cell stage is reached. Thus together the two substances produce an effect that neither alone affects. In another case, LiCl 0.5 per cent. + sugar 1.0 per cent. delayed the closure of the blastopore; a solution of LiCl 0.5 per cent. + sugar 2.0 per cent. also delayed, but did not actually prevent the closure of the blastopore; while a solution of LiCl 0.5 per cent. + sugar 4.0 per cent. stopped the development of most of the eggs in the late cleavage; although in one case at least the outline of the neural plate appeared. The results show that the sugar reinforces the effects (or a part of it at least) of the lithium. It remains now to see whether the results can be explained as due to osmotic pressure alone or whether a chemical effect also occurs.

Concentration.		*Ionization.*	*Osmotic Pressure at 18° C in Atmospheres.*
Per Cent.	Mols. Per Liter.	Per Cent.	
Cane Sugar.			
1	0.029	0	0.698
2	0.058		1.396
4	0.117		2.792
6	0.175		4.188
10	0.292		6.980
12	0.351		8.376
Lithium chlorid.			
0.4	0.094	82.3	3.843
0.5	0.118	82.3	4.804
0.55	0.129	82.3	5.284
0.6	0.141	79.9	5.690
0.65	0.153	79.9	6.161
Sodium chlorid.			
0.3	0.051	87.4	2.15
0.5	0.085	85.2	3.55
1.0	0.171	81.8	6.96
1.5	0.256	79.1	10.28
2	0.342	77.7	13.61
3	0.513	73.7	19.95
4	0.684	71.6	26.28
Lithium chlorid + Sodium chlorid.			
0.4	0.3		5.99
0.5	0.5		8.35
0.5	1.0		11.76
Lithium chlorid + Sugar.			
0.4	5.0		7.33
0.5	1.0		5.50
0.5	4.0		7.59

In the accompanying table, kindly prepared by Dr. H. W. Berg, the osmotic pressures of most of the solutions given in the preceding statement are given. It will be seen for the upper limit of LiCl, namely, 0.65 per cent., the osmotic pressure is 6.161. In comparison the results with NaCl are very different. The upper limit is above 2 per cent. The osmotic pressure for 2 per cent. is 13.61, which is more than double the strength of effective LiCl. The comparison shows that the effects of the lithium salt are not due to osmotic pressure alone.

In a double solution containing LiCl 0.5 + NaCl 0.5 nearly normal embryos appeared, and the osmotic pressure in this case is 8.33, which is much greater than that for the lithium alone. It took, in fact, a solution containing LiCl 0.5 + NaCl 1.0 to prevent gastrulation. The osmotic pressure in this case is 11.76, a pressure far greater than that for the effective limit of LiCl alone.

The upper limit for cane sugar was found to be about 12 per cent., which corresponds to an osmotic pressure of 8.376. This is much higher than for the lithium chlorid alone.

For solutions of LiCl 0.4 + sugar 5.0 per cent., only the 64-cell stage was reached. The osmotic pressure is 7.33, which is again much higher than for lithium chloride alone, but less than that for the sugar alone. It would seem, therefore, that the two together must have a higher pressure than for the one producing its effects at the lower limit, but less than for the other that produces its effects at a higher pressure. Similar conclusions might be drawn from the double solution containing LiCl 0.5 + sugar 4.0 per cent. The results show that there is a double effect produced by salt solutions, a chemical and an osmotic. How each effect is produced we do not know at present. Loeb has shown, however, that living substances behave very differently towards the amount of water absorbed according to what chemical element, sodium, calcium, or lithium, for example, that they have taken up. Since the development of the embryo is associated with the amount of water absorbed it might appear that in this way the chemical action is similar to the results produced by means of osmotic pressures which also effect the amount of water. That the egg contains enough water in itself for normal development is shown by my

experiments of causing it to develop out of water, but this does not show that if water is removed beyond a certain point or the capacity to absorb water is changed the development might not be delayed. In fact if the egg is dried too much it fails to develop. However this may be, the point of special interest brought out by these experiments is that an effect may be produced by a double solution in which the total osmotic pressure is lower than that required to produce the effect by one of the substances alone, but higher than that sufficient to produce the result by the other alone. It seems probable that the effect is a double one ; in part chemical, in part osmotic.